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Interstitial Diode Laser Hyperthermia in the Treatment of Subcutaneous Tumor

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Background and Objective: **Interstitial Laser Hyperthermia (ILH) has been investigated since the early 80s in the treatment of deep seated tumors. The purpose of this study was to evaluate the efficiency of diode ILH (830 nm) in a subcutaneous tumor model.**
Study Design/Materials and Method: **The antitumoral effect of diode ILH was assessed in a randomized study performed on 80 Swiss nu/nu mice. The tumor model was a subcutaneously implanted HT29 colonic carcinoma. The animals were assigned to four groups of 20 mice: Groups 1 and 3 were treated by ILH, groups 2 and 4 were not treated (control groups). Tumors were removed on day 3 in groups 1 and 2, and on day 30 in groups 3 and 4. The treatment was performed on tumors of 8 mm in diameter and a volume of 140 mm^3. A laser irradiation of 360 J (power: 0.2 W; irradiation time: 1 800 s) was delivered through a 300 μm optical fiber implanted in the tumor. The laser parameters insured temperatures of 46°C in the central part of the tumor and 42°C at the periphery. Tumor features were evaluated on day 3 and day 30.**
Results: **Untreated tumors grew rapidly up to a mean volume of 241 mm^3 on day 3 (group 2) and 2,000 mm^3 on day 30 (group 4). Treated tumors regressed to a mean volume of 32 mm^3 on day 3 (group 1). On day 30, 40% of the tumors had totally disappeared and 60% showed partial response with small and peripheral residual tumor of 172 mm^3 on an average, as to say 11.2 times smaller than in group 4.**
Conclusion: **ILH with a low power 830 nm diode laser is an efficient treatment of subcutaneous tumor model. Partial responses are attributed to an insufficient heating at the tumor periphery. More precise control of the peripheral tumor temperature will improve the ILH results.**

Key words: diode laser, human colonic cancer, interstitial hyperthermia, tumor cell kill

INTRODUCTION

Interstitial Laser Hyperthermia (ILH) is a new approach in the palliative therapy of cancer proposed by Bown in 1983 [1]. The aim of this procedure is to destroy deep seated tumors in situ by localized laser heating [2,3]. Experimental evaluation of ILH has been performed using low power irradiation delivered through optical fiber into normal tissue [4] and subcutaneous tumors [5]. In solid organs the fiber was interstitially implanted into tumors under ultrasound [2,6]. A

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well demarcated area of coagulative necrosis around the fiber tip while sparing adjacent tissue have been reported [4,7]. In any case, investigators have demonstrated the interest of the procedure in the palliative treatment of human hepatic and brain tumors [2,3,6,8]. Initially the Nd:YAG laser (1,064 nm) has been preferred because of its high optical penetration in soft tissues [1,4,7]. However technical drawbacks of this laser were multiple: cumbersome size, fixed installation due to water cooling and to power supply, as well as expensive maintenance. In order to avoid these disadvantages some authors have proposed the introduction of diode laser in this application [9,10,11]. Three characteristics advocate for its use in ILH: good penetration of its wavelength in tissue, small size of the device, and easy transmission through a flexible quartz fiber [9,10,11].

The purpose of this paper is to evaluate the potential of a 830 nm diode laser in the treatment of a subcutaneous tumor model implanted in Swiss nu/nu mice. Thermal effects, tumor volume reduction, microscopic changes as well as tumoral recurrence rate after a 30 day survival period, are reported.

MATERIALS AND METHODS

The tumor model used was a HT29 human colonic carcinoma obtained from the Alexis Vautrin Center (Nancy, France). Tumor grafts (1 mm in diameter) were subcutaneously transplanted into the left back side of mice anaesthetized by intramuscular ketalamine (100 mg/kg). Eighty nu/nu Ico: Swiss mice-32 males and 48 females, 6 weeks old, 25 g in weight (Iffa Credo, l'Arbresle, France), housed in desinfected cages and maintained at an ambient temperature of 26°C, have been inoculated.

ILH was performed with a 830 nm diode laser source. The prototype, realized at the Laboratoire d'Optique Appliquée (EPFL, Lausanne, CH, Switzerland), was of small size (20 × 36 × 45 cm and 13 kg) and provided a continuous wave beam. Its output was coupled into a 300 μm core diameter optical fiber with a numerical aperture of 0.2. Up to 3 W laser power was available at the tip of the fiber. Laser tissue irradiation was performed by implanting the bare distal fiber tip in the center of the tumor. The laser power delivered into the tumor was 0.2 W and the exposure time 1,800 s. The laser parameters were chosen in order to obtain a temperature of 46°C at 3 mm from the fiber tip.

The treatment was applied in tumors of about 8 mm in diameter, corresponding to a tumor volume (TV) of about 140 mm^3 (day 0). The size of the tumor was limited because of the small size of animal and to allow a single fiber irradiation procedure. The mice were randomly assigned to four different groups (n = 20 for each group). Groups 1 and 3 were treated by diode ILH with the irradiation parameters above. No laser treatment was performed on groups 2 and 4 (control groups). Tumors were removed and their size measured on day 3 (groups 1 and 2) and on day 30 (groups 3 and 4). In groups 1 and 2, the recurrence rate was evaluated on day 30. The TV of each tumor was determined assuming an ellipsoidal tumor shape. The TV was calculated according the formula: $TV = 4/3\ \pi\ hd1d2$ (h, d1 and d2 being respectively half height, half largest diameter, and half smallest diameter). The quantitative data were compared using Kruskal-Wallis, Chi. square, student's *t*-tests, as well as analysis of variance. The level of statistical test was $p = 0.05$.

During the treatment the mean temperature at 3 mm from the fiber tip and at the periphery of the tumor were simultaneously evaluated by K thermocouples, (Bioblock Scientific, Illkirch, France) connected to a digital thermometer, (Bailey Instruments Inc., Saddle Brook, NJ). The characteristics of the thermocouples were a sensitivity of 0.1°C and a response time of 0.3 s.

Removed tumors and recurrence controls fixed in Bouin Holland, were prepared in paraffin sections (7 μm thick), and stained with haematoxylin eosin or Heidenhain's azan.

RESULTS

Clinical Observations and Tumor Volume

On day 0, the tumors of the different groups were regularly ellipsoid with a soft consistence. The overlying skin presented no special adhesion nor anormal vascularization. The mean TV was respectively 140 ± 38 mm^3, 135 ± 49 mm^3, 142 ± 27 mm^3, and 134 ± 27 mm^3 in group 1, 2, 3, and 4. No significant difference was observed between the four groups ($F = 0.2262$, $p > 0.5$). After treatment, a small edema and whitening of the skin overlying the tumor could be observed. The temperature measured during irradiation was 46°C at the central part of the tumor and 42°C at the periphery, while the mice body temperature was 37°C.

On day 3, macroscopic modifications have

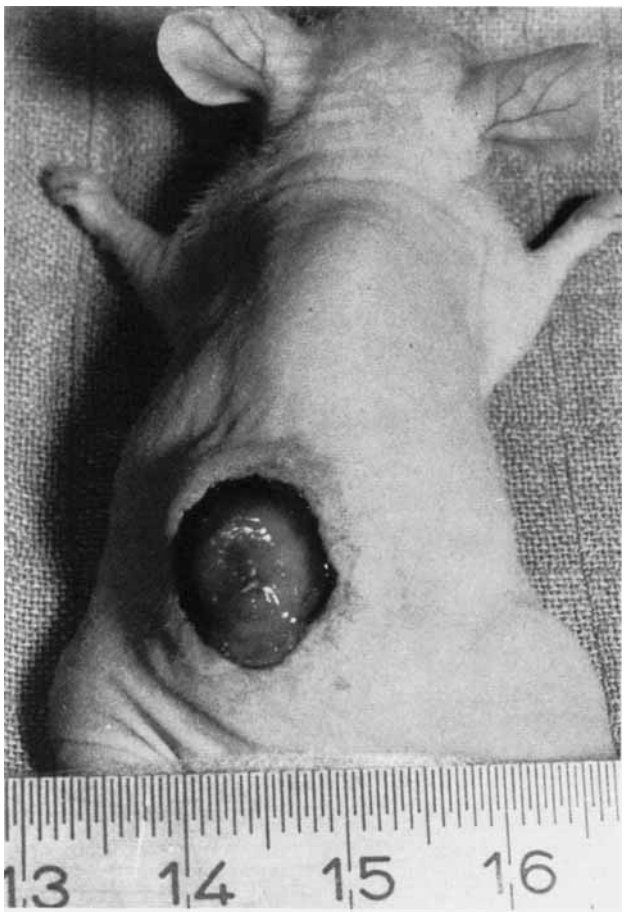

Fig. 1. Macroscopic aspect of HT29 colonic carcinoma on day 3 after ILH (group 1). The tumor is shrunken and retracted by heating effect.

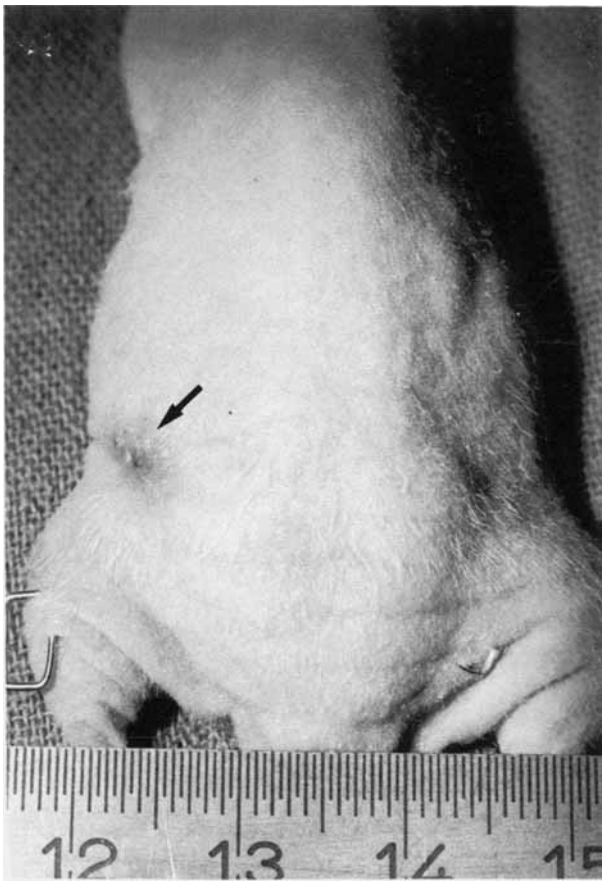

Fig. 2. Macroscopic aspect of the curing on day 30 after ILH (group 3). The skin scar is entirely free of tumor (black arrow).

been observed through skin incision. In the treated group (group 1) the TV decreased considerably to a mean volume of 32 ± 15 mm^3 ($t = 11.93$, $p < 0.001$). The external aspect of the tumors was whitish and retracted, and seemed deprived of vascularization (Fig. 1). By contrast in the control group (group 2) the tumor were cauliflower-like significantly increased in size, mean TV 241 ± 70 mm^3 ($t = 5.55$, $p < 0.001$). There was a significant difference in TV between groups 1 and 2 ($t = 3.60$, $p < 0.001$). Finally, on day 30 the recurrence rate of group 1 was 20% vs. 55% for group 2 ($\chi^2 = 10.9$, $p < 0.02$).

On day 30, treated tumors (group 3) had completely disappeared in 40% of cases (Fig. 2). In the remaining 60% a partial response was observed with a residual tumor corresponding to a mean TV of 179 ± 109 mm^3. Comparatively, in the control group (group 4) TV was increased to 2,000 ± 1,294 mm^3 ($t = 6.4$, $p < 0.001$) (Fig. 3). At day 30, the volume of treated tumors was 11.2 times smaller than in the control group. The difference between TV of groups 3 and 4 were significant ($t = 6.2184$, $p < 0.001$).

Microscopic Observations

On day 3, the microscopic examination of tumors of group 1 (ILH treatment) showed extensive and complete necrosis of the tumor tissue (Fig. 4). The axis of the tumor was occupied by an oblong central area of cavitation with a thin layer of carbonization due to the optical fiber positionning and to the light track. In the surrounding inner zone there was coagulation necrosis, tumoral cells were totally destroyed. The outer zone presented edema and cellular shrinkage without any viable tumor cells. At the periphery there was a clear limit occupied by a line of dark pycnotic and necrotic debris underlined by a high density of inflammatory cells. Nevertheless in two cases, peripheral foci of viable tumoral cells

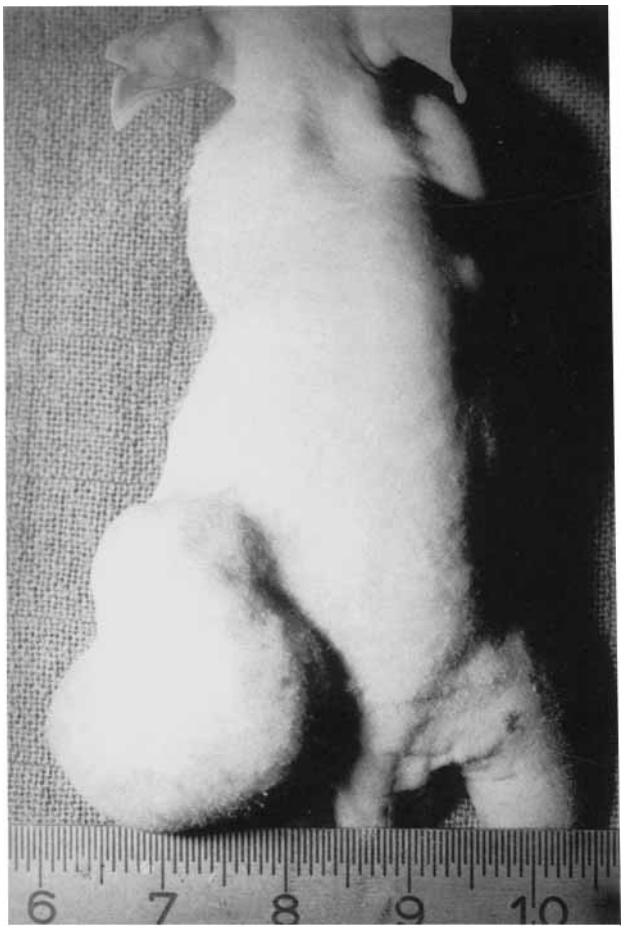

Fig. 3. Tumor control (group 4) on day 30.

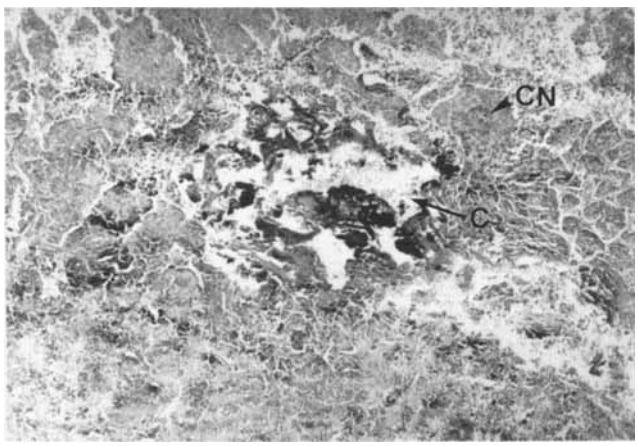

Fig. 4. Microscopic aspect of a ILH treated tumor on day 3 (group 1). C, crater, CN, coagulation necrosis. HE × 46.

were found in lateral tumor extensions. In group 2 (control group) the microscopic examination visualized in any case a tumor densily occupied by active cancer cells (Fig. 5).

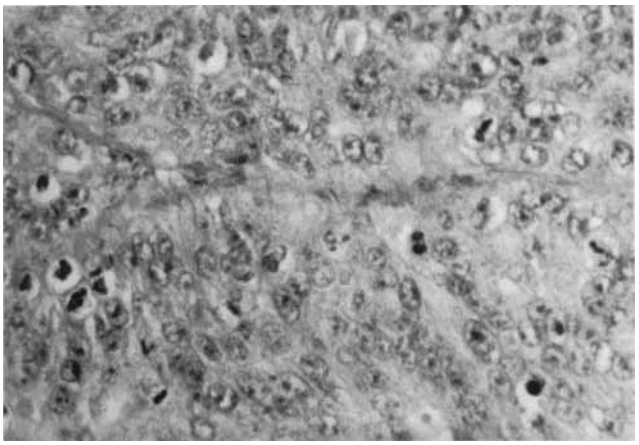

Fig. 5. HT29 tumor tissue in control group on day 3 (group 2). Note the viable tumors cells with numerous mitosis. HE × 460.

On day 30, group 1 recurrences presented some small areas of necrosis scattered between tumors cells. In group 3, the microscopic examination showed in 40% of the cases a fibrotic scar with inflammatory cells and neovascularization without tumor cells (Fig. 6). In 60% of the cases, foci of tumoral recurrences could be detected in the peripheral part of the tumor, surrounding the central area occupied by fibrotic tissue free of tumoral cells.

DISCUSSION

The aim of the study was the evaluation of the curing potential of 830 nm Induced Laser Hyperthermia (ILH) into a small tumor model implanted in mouse. The main reason of the choice of a small tumor model was driven by our concern to restrict the study to a single irradiation procedure. Larger tumors need multiple fiber applicators or multiple irradiation procedures, which increase the risk of artifacts and complicate the interpretation of the results.

The irradiation conditions were determined in order to minimize tissue alteration in the immediate vicinity of the fiber tip, to insure efficient light penetration into the tumoral tissue during the whole procedure, avoiding excessive heating in the center of the tumor, tissue vaporization, disruption (popcorn effect), and tissue charring. Although a significant recurrence rate was observed, efficient tumoral volume destruction was demonstrated at the chosen irradiation condi-

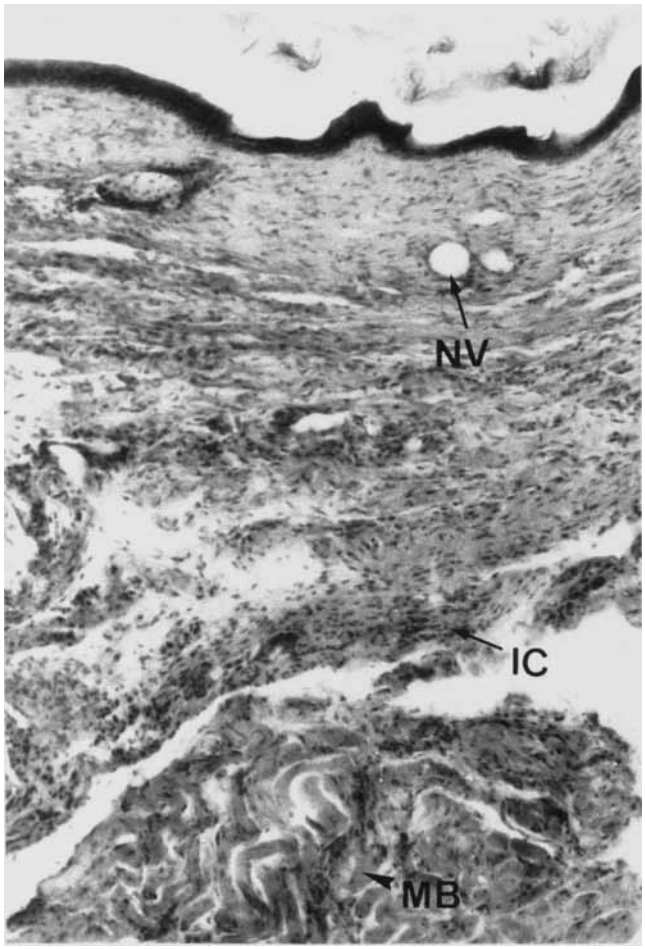

Fig. 6. Microscopic aspect of subcutaneous healing process on day 30 after ILH (group 3). Note inflammatory cells (IC), muscular bundles (MB) and neovascularization (NV). HE × 110.

tions: laser power 0.2 W and irradiation time 1,800 s. In 40% of the cases, complete tumor destruction was confirmed 30 days post-operatively. In the remaining 60% only peripheral and limited recurrences were observed. In any way, the mean recurrence volume was significantly reduced by a factor of 11.2 compared to untreated tumors.

The necrotic lesions were well demarcated from the surrounding normal tissue. The lesions evidenced a characteristic architecture, which consisted in a central crater resulting from the fiber insertion and tissue vaporization immediately surrounded by an intermediate zone of coagulation necrosis and by a peripheral edema scattered by inflammatory cells. Tissue alteration features were comparable to that observed after Nd:YAG irradiation of normal tissue [4,7,12] as well as after diode laser application [9]. Charring in the center of the tumor indicated that even at the low power irradiation (0.2 W) and an irradiance value of 283 W/cm^2 at the fiber tip tissue temperatures higher than 100°C were reached in the immediate fiber vicinity. Simultaneously, tumor recurrence proved insufficient tissue heating at the periphery.

These data well illustrate the limits of ILH. According to the results, the chosen tumor size was too large to insure a complete curing of the tumor in a single irradiation procedure. Recurrence resulted from inefficient temperature control at the periphery of the tumor, although water boiling temperature were reached at the fiber tip. Obviously, complete temperature control at the tumor periphery is of paramount importance in ILH procedures. Complete peripheral temperature control is impaired by the inhomogeneity of the tumor architecture and by the local cooling effect of the peripheral blood supply.

The recurrence rate of 20% observed by day 30 in group 1 is probably due to an insufficient heating effect on the polycyclic tumors, or to an intense vascular cooling effect. To insure peripheral tumor destruction temperatures superior to 46°C have to be applied. As we currently do in surgical resections, a supplementary rim of normal tissue beyond the limit of the tumor has to be treated by laser hyperthermia.

In order to achieve ILH tumor treatment, the tumor size and architecture have to be carefully determined. Both informations have to be taken into account to define the irradiation parameters and particularly the number of irradiation sites. The optical properties of the tumoral tissue determine the choice of the suitable laser irradiance to apply [5]. The peripheral vasculature has to be considered as well.

Simultaneous firing of multiple fibers or several irradiations with a single fiber might optimize the ILH effect but placing fibers remains difficult to organize, and the result difficult to predict. Another possibility is to increase the laser irradiance with the risk to majorate carbonization and then to accept to modify light transmission in tissue. This procedure could be proposed as interstitial thermotherapy in order to obtain more effective tumor destruction.

In conclusion, this work shows the partial efficiency of ILH with low irradiance and long time duration and proves the limits of the procedure against polycyclic tumors with dense peripheral blood supply. Further exploration should con-

cern increased irradiation and will be presented in a paper to come.

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